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Observations on the Fine Structure of the Stichosome and Bacillary Band of Trichuris Muris (Schrank, 1788) and Trichuris Vulpis (Froelich, 1789).

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OBSERVATIONS ON THE FINE STRUCTURE OF THE STICHOSOME AND BACILLARY BAND OF TRICHURIS MURIS (SCHRANK, 1788) AND TRICHURIS VULPIS (FROELICH, 1789)

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ABSTRACT

The structure of the stichosome and bacillary band of the members of the superfamily Trichuroidea has been described by several investigators. However, the exact morphology and function of these organs have not yet been determined. This electron microscopy study has been made to clarify the structures previously defined by light microscopy and to attempt to relate the fine structure to cellular function.

Adult Trichuris muris and T. vulpis were obtained from their respective hosts with induced or natural infections. The anterior portion of the worm was straightened on dry filter paper and fixed for 30 minutes in buffered osmic acid with sucrose added. The tissue was then dehydrated and embedded in Selectron-methacrylate or butyl-methyl methacrylate. Sections were cut, mounted on grids, stained in some cases and viewed with the electron microscope.

The bacillary band is composed of a large number of tall slender cells. The basal end of the cell rests on a thick basement membrane. The basal plasma membrane of the cell is folded and interdigitates with adjacent cells. Occasionally, a large homogeneous area containing glycogen is seen in the basal end of the cell. The cytoplasm of the basal portion of the cell is filled with RNP particles and a fine endoplasmic reticulum. The Golgi apparatus consists of many small vesicles near the basal end of the nucleus. The nucleus is ellipsoidal and enclosed by a double membrane. The dense nucleolus

is central or subcentral. The area apical to the nucleus contains numerous mitochondria and a complex system of lamellae. These lamellae are formed by invaginations of the plasma membrane at the apical end of the cell. A homogeneous secretory mass is adjacent to the lamellae and fills the cuticular pore.

The stichosome consists of a row of cells each of which completely or partially encloses the esophagus. Each stichocyte is longer than broad and has several infoldings of the lateral margins, giving an appearance of several cells. The entire stichosome is enclosed by a dense thick membranous structure consisting of many fine membranes. The plasma membranes separating the stichocytes are irregular and the cells are loosely interdigitated. The spherical nucleus is central and contains a large dense vacuolar nucleolus. The nuclear membrane is regular in gross appearance but has many fine blebs arising from its surface. Numerous electron-dense secretory granules are found scattered in the cytoplasm. Mitochondria are dispersed among the granules and are numerous along the outer margin of the cell. Large vesicles with connecting canaliculi are distributed throughout the cell. The remaining cytoplasm is filled with endoplasmic reticulum consisting of vesicles and lamellae. The esophagus is also enclosed in the membranous structure previously mentioned. The esophageal lumen is lined with cuticle and usually appears hexaradiate. The tubular

esophageal cell encloses the lumen. Occasionally myofibrils perpendicular to the lumen are seen in the esophageal cells.

The gross structure of the stichosome and the bacillary band as observed by electron microscopy is generally in accord with that described by light microscopy. The fine structure indicates the stichocytes to be secretory in function, but no evidence of a secretion leaving the cell has been observed. The bacillary band also appears to secrete a product through the cuticular pores but the nature of this product is undetermined. Studies of the cyclic behavior of these cells may help relate their morphology and functions.

I. INTRODUCTION

The stichosome and bacillary band of the anterior portion of the members of the superfamily Trichuroidea have been studied by many investigators regarding their exact morphology and function. Eberth (1859) undertook the first serious microscopic study of these structures in Trichuris trichiura. He noted, on the ventral side of the worm, the presence of a granular band ("Stäbchenfeld" or bacillary band of later authors) which consisted of many rod-like shafts. He described the esophagus as being embedded in a row of large cells which he termed "Zellenkörper" (cell body or stichosome of later authors) and assigned a glandular function to it. Bastian (1866) interpreted the bacillary band as an area of closely packed cuticular pores. He described the esophagus as a cylindrical organ with a central, oval lumen and considered the usual esophageal muscle fibers to have been almost wholly replaced by large nucleated cells with granular contents. Leuckart (1876) also recognized the ventral granular band. He considered the cell body to surround the esophagus, both being enclosed in a peritoneal membrane. He suggested that the cell body was an outgrowth of the dorsal esophageal wall.

Jägerskiöld (1901) studied Cyclicolaimus magnus, Thoracostoma acuticaudum and Trichosomum obtusiusculum. He found that these nematodes and probably others were richly provided with hypodermal

glands, each of which opened through a pore of the "Stäbchenfeld". The usual excretory organs were absent. On the basis of these observations he proposed that the hypodermal glands were excretory in function. Müller (1929) considered the intestine to be excretory in function with the "Kopfdrusen" [hypodermal glands of Jägerskiöld (1901)] and the cell body playing an important role in the nutrition of the organism. He thought the glands of the bacillary band produced a secretion which dissolved the host tissue externally. Subsequently, the digest was absorbed osmotically through the cuticular pores of the glands. He thought the cell body served an osmotic function either by a secretion or by its contractile fibers which connected it to the bacillary band.

Rauther (1918) made a detailed study of the bacillary band and the cell body. He disagreed with Jägerskiöld's use of the term "Stäbchenfeld" since the "Stäbchen" was actually a secretory product of the underlying cell. Previous workers had not noticed that the rod-shaped structures of the bacillary band consisted of these two parts. In studying the structure of the cell body, Rauther (1918) reported the presence of intracellular canals as had Eberth (1859) and Bastian (1866). These canals were observed to open through pores into the capillary esophagus in the vicinity of the nucleus.

Müller (1929) thought the esophagus to be non-functional as a feeding organ since it lacked muscle fibers. He considered the radial fibers of the esophagus, called "dilatatores oesophagi" by

Heine (1900), to be part of the basal membrane. Müller's theory was rejected by Chitwood (1930) who found small radiating muscle fibers in the esophageal wall. In a later work, Chitwood (1935) reported the presence of an opening into the esophageal lumen in each cell of the stichosome. This strengthened his opinion that the cells, which he termed "stichocytes", were esophageal glands.

The foregoing investigations employed light microscopy. With the introduction of the electron microscope, however, it became possible to study the fine structure of cells, clarify previous descriptions and add many new concepts regarding function. At first observation was limited to shadowed particulate matter. Later, the introduction of butyl methacrylate embedding by Newman et al. (1949) and polyester embedding by Kellenberger et al. (1956) in combination with the Porter-Blum or Sjöstrand microtomes made it possible to cut routinely thin sections of tissues. Osmium tetroxide was determined to be the general fixative of choice in comparative studies by Dalton et al. (1950) and Pease and Baker (1950). Palade (1952) recognized the need for more consistent results and combined osmium tetroxide with an acetate-veronal buffer. This fixative was then modified by Caulfield (1957) by the addition of sucrose to increase the tonicity to that of the particular tissue.

Using these basic techniques and later modifications, electron microscopists have examined the fine structure of cells. The delicate nuclear membrane of light microscopy was found to be a double

membrane with numerous pores by Callan and Tomlin (1950) in their studies of amphibian oocytes. Wischnitzer (1958) observed that the nuclear pore consisted of a cylinder made up of eight microcylinders in the immature oocytes of Triturus viridescens. Anderson and Beams (1956) reported the transfer of nuclear contents into the cytoplasm through the nuclear pores. Bennett (1956) proposed that the RNP particles produced in the nucleus were carried into the cytoplasm by being attached to the inner nuclear membrane which flowed outward at the pore thus becoming the outer membrane. A recent work by Clark (1960) described the presence of nuclear blebs in pancreatic acinar cells of the rat. He reported that the blebs might indicate a mechanism of transference of nuclear material into the cytoplasm. Watson (1955) studied the nuclear membrane of a variety of mammalian cells and described many large pores where the nucleoplasm and cytoplasm were continuous with no membrane separating them as reported by Afzelius (1955). Watson (1955) and Palade (1955b) considered the outer nuclear membrane to be rough-surfaced endoplasmic reticulum and the space between the inner and outer membranes to be continuous with the cavities of the endoplasmic reticulum.

The endoplasmic reticulum was first observed by electron microscopy by Porter et al. (1945). They described a lacelike reticular structure in the cytoplasm of tissue culture cells. Later, Palade and Porter (1952) studied the endoplasmic reticulum in sectioned tissue and reported the system to have various forms from isolated

vesicles to networks of canaliculi. Gay (1956) reported nuclear outfoldings which seem to contribute to the formation of endoplasmic reticulum. Kurosumi (1956, 1958) concluded from observations of mitosis in sea urchin blastomeres that the nuclear membranes might be replaced by endoplasmic reticulum at telophase. Amano and Tanaka (1957) and Yasuzumi (1959) reported that the endoplasmic reticulum encloses the chromosomes and becomes the nuclear envelope at the end of mitosis. The granular nature of the endoplasmic reticulum membrane was first noted by Weiss (1953) and later the granules were described as attached or free in the cytoplasm by Palade (1955a). Palade and Siekevitz (1956) demonstrated that the granules were rich in ribonucleic acid. In their studies of cytoplasmic membranes, Dalton and Felix (1953) found an area of smooth surfaced membranes in the cytoplasm which corresponded to the Golgi apparatus of light microscopy. They found it to consist of lamellae, vacuoles, and granules bounded by smooth dense membranes.

Secretory cells have been extensively studied by electron microscopy. Dalton (1951) reported the secretory granules of pancreatic exocrine cells as large spherical granules of high electron density. The basal cell surface of some secretory cells has been shown to be highly folded. Pease and Baker (1950) found "tubular sheaths" surrounding the mitochondria in the proximal convoluted tubule. Pease (1955) later described the sheaths as infolded basal plasma membranes. He suggested that the infolding was related to the trans-

port of water. Weiss (1953) first noted basal infoldings in pancreatic acinar cells. Subsequently, structures of this type were noted in many cells which were concerned with absorption or secretion.

Mitochondria were observed by various investigators but the internal structure was beyond the limits of resolution of light microscopy. Palade (1952) began the investigation of fine structure of mitochondria. He described the mitochondria as being surrounded by an outer and inner membrane, the latter folded inward forming ridges in the mitochondrial cavity. He termed these ridges "cristae mitochondriales". Lehninger (1956) and Green and Hatefi (1961) reported the fundamental functions of mitochondria to be electron transport along with coupled phosphorylation and secretory activity. Challice and Lacy (1954) postulated that secretory granules in the pancreas might originate from mitochondria. Fawcett (1954) found suggestions of splitting of mitochondria and thus increasing their number. However, Rouiller and Bernhard (1956) thought they were formed from microbodies in hepatic cells.

A comparison of electron microscopic studies with previous light microscopy investigations should clarify and expand the present knowledge of the structure of bacillary cells and stichocytes of the genus Trichuris. Comparison of the fine structure of these cells with that already reported in the literature described above may give an insight into the relationship of form to function in the nematodes under investigation.

II. MATERIALS AND METHODS

Trichuris muris

T. muris adults used in this study were obtained from laboratory infected DBA mice. Studies by Worley *et al.* (1960) have shown that the DBA-2 strain is the most susceptible to infection. The method used in maintaining infected mice is a modification of that described by Fahmy (1954) and Worley *et al.* (1960).

Eggs were obtained by two methods: 1. Fecal pellets were collected on moist paper toweling overnight. The following morning the pellets were emulsified in tap water and allowed to sediment. It was necessary to wash the sediment repeatedly to remove fecal debris and food particles. 2. The other method of obtaining eggs was by maceration of adult female worms. About ten worms were placed in a conical test tube and macerated in a small volume of tap water with two wooden applicators. After the worms were sufficiently fragmented, the pieces were repeatedly drawn up and expelled from a 1 cc syringe to remove the eggs from the uterus. The eggs were then washed and allowed to settle prior to culturing. Eggs obtained by either collection method were cultured at room temperature (25°C) in 0.85% saline with 1% formalin to inhibit bacterial growth. About 35 days were required for complete embryonation. The collection of eggs by maceration of the female worms was the more desirable method. Although the percent yield of embryonated eggs was lower with this method, the total number of embryonated eggs obtained

was higher. The repeated washings and the inability to remove all of the debris render the fecal collection method less desirable.

The eggs were washed several times to remove the formalin prior to inoculation of the mice. Three samples of 0.1 ml each were counted to determine the number of embryonated eggs present in the culture. The volume of the culture was then adjusted to provide 150 to 200 eggs per mouse when a 0.1 ml aliquot was inoculated. Weanling mice, about three to four weeks old, were inoculated orally by introducing a blunt 20 gauge needle. Ether anesthesia was used to prevent struggling and possible damage to the esophagus.

Maturation of the worms required approximately 35 days. The mice were sacrificed and the ceca removed to a petri dish containing 0.85% saline and teased open with dissecting needles. Since the anterior portion of the worm is embedded in the intestinal mucosa, the posterior end must be grasped with forceps and gently pulled until the worm is free. The worms were then washed in warm saline.

Trichuris vulpis

T. vulpis specimens were obtained from mature stray dogs after sacrifice. The ceca were removed and carefully opened with scissors. The worms were removed from the mucosa as described above. They were then washed with warm saline prior to fixation.

Fixation

Fixation was carried out on filter paper in a petri dish cooled in an ice bath. The worms were straightened on dry filter paper and

immediately bathed in cold fixative. This technique prevents coiling of the anterior end. After several minutes the worms were transferred to a flask of cold fixative.

Buffered osmic acid of Palade (1952) with sucrose added (Caulfield, 1957) was used as a fixative in this investigation. Prior to use, the fixative was cooled in an ice water bath to approximately 4°C. Tissues were fixed for 30 minutes. Tissues to be embedded in Selectron-methacrylate were removed from the osmic acid fixative, washed with distilled water and placed in 10% buffered neutral formalin for 12 hours.

Tissues were washed in distilled water after fixation and dehydrated with either ethyl alcohol or acetone. Concentrations of 35, 50, 70, 85, 95, and 100% ethyl alcohol were used for 30 minutes each. Three changes in 100% ethyl alcohol filtered over Drierite were used to insure removal of all water from the tissues. Concentrations of 35, 50, 70, 80, 90, and 100% were used for 30 minutes each when acetone was the dehydrating agent. Three changes of anhydrous 100% acetone were used to insure adequate dehydration.

Embedding

After dehydration, the tissues were placed in a 1:1 mixture of the anhydrous dehydrating agent and the embedding medium for one hour. Tissues were then transferred to pure uncatalyzed embedding medium for one hour, followed by transfer into catalyzed embedding medium.

Two types of embedding media were used. Tissues fixed in osmic

acid and dehydrated in alcohol were embedded in a 3:1 mixture of butyl and methyl methacrylate. Benzoyl peroxide (1.25% was used as a catalyst. Polymerization was carried out at 60°C. for 16 to 24 hours. Tissues fixed in osmic acid, post-fixed in neutral formalin and dehydrated in acetone were embedded in a 1:1 mixture of Selectron and butyl methacrylate according to the method of Low and Clevenger (1962). This mixture was catalyzed by tertiary butyl hydroperoxide (0.5%) and polymerized for 24 hours at 60°C.

Sectioning

Sections were cut with glass knives on a Porter-Blum microtome or an LKB Ultratome. Those sections which had pale gold, silver or gray interference colors were used. The sections were expanded carefully with xylene vapors before being picked up on grids. Methacrylate sections were picked up on 200 mesh 0.25% formvar-coated copper grids. Selectron-methacrylate sections were mounted on 400 mesh copper grids without a supporting membrane.

Selectron-methacrylate sections were heat and solvent cleared to produce better stability in the electron beam and to increase the contrast of the image (Low, 1960). Heat-clearing was accomplished by placing the grids in a cool electric furnace and gradually bringing the temperature up to 225°C. over a period of at least one hour. This temperature was maintained for two hours after which the grids were allowed to cool. Sections were then solvent-cleared in a 1:1 mixture of acetone and ethyl ether for 30 minutes. Staining of some

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sections was done by adding several small crystals of uranyl nitrate to the solvent mixture. After staining, the sections were rinsed with pure solvent mixture and allowed to dry.

Electron microscopy

Sections one to two microns thick were cut either before or after thin-sectioning for orientation. They were removed from the knife trough and placed on a glass slide in a drop of immersion oil, covered with a coverglass and viewed with a phase microscope.

Thin sections were viewed in an RCA EMU-2 electron microscope. Photographs were made on 2x10 Kodak Lantern Slide Plates (Medium Contrast) and developed for 8 minutes in Ansco Hyfinol Developer. Prints were made on Kodak Kodabromide paper.

Light microscopy

Tissues were fixed in AFA or in Carnoy's fixative for 18 hours. Dehydration was carried out in ethyl alcohol. The tissues were embedded in paraffin and sections cut six microns in thickness. The sections were stained with hematoxylin and eosin for the purposes of orientation. Best's carmine or Toluidine blue were used for histochemical studies, the results of which were inconclusive.

Measurements

Random measurements were made of individual structures in the micrographs and average sizes computed. Measurements in millimeters were made with a calibrated magnifier and then converted to angstroms according to the magnification of the micrograph.

III. RESULTS

The following results were derived from the study of tissue obtained by random sampling from the middle portion of the whip-like anterior end of T. muris and T. vulpis. Unless otherwise stated all data given apply to both species.

The bacillary band

The bacillary band is an area of cuticular pores and underlying hypodermal gland cells. It begins a short distance from the anterior end of the worm and extends to the enlarged posterior region. In cross-section the bacillary band occupies from 1/4 to 1/3 of the circumference of the worm. The cuticle of this area has three distinct layers. The outer layer is approximately 0.6 to 0.7 micron in thickness and has numerous invaginations from 0.2 to 0.4 micron in depth. The middle layer has a greater electron density and is from 0.3 to 0.4 micron in thickness. It is a smooth surfaced layer. In the vicinity of the cuticular pores the middle layer is interrupted and does not separate the inner and outer cuticular layers. The inner layer has the same amorphorous appearance as the outer layer and is 0.45 to 0.55 micron in thickness. The cuticular pores are generally conical. They are widest at the base of the inner cuticular layer and taper to their narrowest point at the level of the middle layer. The opening through the outer cuticular layer is usually slightly wider than that of the middle layer. The pore

diameter at its narrowest point is approximately 1.5 microns. The pore area is occupied by a plug of material secreted by the underlying cell (Figs. 1, 2).

The bacillary cells are of hypodermal origin and are formed by enlargement of one of the lateral lines of the worm (Fig. 3). They are elongate cells which lie perpendicular to the cuticle and extend from it to the pseudocoelomic cavity. The basal end of the cell rests on a thick dense basement membrane approximately 750 Å in thickness (Fig. 4).

The plasma membrane of the bacillary cell is 70 Å in thickness (Fig. 6). At the basal end of the cell the plasma membrane has numerous foldings allowing interdigitation of adjacent cells (Fig. 4). The lateral plasma membranes are relatively straight. They are usually separated from each other by a 100 Å space (Fig. 6). No terminal bars, desmosomes, interdigitation or other means of cell attachment were observed. The apical edge of the cell has numerous deep invaginations which extend approximately 4 microns into the cell (Figs. 1, 6). These invaginations form a system of lamellae which greatly increase the surface area of the cell. The lamellae are about 400 Å in thickness and are separated by a space of equal magnitude. Vesicles often separate the lamellae at their proximal end (Fig. 1).

The mitochondria of the bacillary cells are pleomorphic (Figs. 6, 7, 8). They have an average diameter of 0.25 micron and range

from 0.25 to 2 microns in length. They lie parallel to the long axis of the cell and are dispersed between the lamellae and at their proximal ends. The interior of the mitochondrion contains a homogeneous electron-dense material and numerous cristae. The cristae appear to be tubular rather than lamellar and often lie parallel to the long axis of the mitochondrion (Fig. 8). The average diameter of the cristae is 100 Å.

The nucleus of the bacillary cell is ellipsoidal in shape and is usually in the center of the cell (Fig. 5). It is approximately four microns in length and from one to two microns in diameter. The nuclear envelope is regular in outline and consists of two membranes. Discontinuities in the nuclear envelope have been observed. Whether they represent nuclear pores or are merely artifacts of fixation has not been determined. The nucleoplasm is a homogeneous area of fine granules with moderate electron density. The nucleolus is central or subcentral in position and often close to the lateral wall of the nucleus (Fig. 9). It is approximately 0.35 micron in diameter and spherical in shape.

The cytoplasm of the basal region of the bacillary cell contains many small granules ranging from 75 to 170 Å in diameter (Fig. 9). The majority of the granules have no noticeable orientation. Granules are occasionally seen in two parallel rows separated by a space of 200 Å. These granules are believed to be the RNP particles which are attached to the membranes of the endoplasmic reticulum. The ER membranes are very fine and are rarely observed in longi-

tudinal section. In cross sections the cavities of the endoplasmic reticulum appear as fine vesicles about 150 Å in diameter (Fig. 9). Adjacent to the nucleus there is a cluster of small vesicles which may be the Golgi apparatus of the cell (Figs. 10, 11). In some cells there is a large area devoid of the above RNP-like particles but filled with a homogeneous fine particulate substance (Fig. 5). This substance appears to be identical to that described as glycogen by other authors (Beckett and Boothroyd, 1961).

A small number of very dense granules ranging in size from 0.04 to 0.1 micron is infrequently observed in the bacillary cells of T. vulpis (Fig. 10). The granules are found in the cytoplasm basal to the nucleus. In this same region spherical bodies up to 1.5 microns are seen. These bodies consist of a dense wall enclosing from 1 to 8 or more vacuoles which have no internal structure. They are usually seen close to the vesicles previously described as the Golgi apparatus. Their structure and location suggest that they are lipochondria. Neither osmophilic granules nor lipochondria have been observed in the bacillary cells of T. muris.

The stichosome

The stichosome consists of a single row of large cells which enclose or partially enclose the esophagus. The lateral edges of the cells are invaginated at several places giving it an appearance of being segmented. The stichocyte is enclosed by a plasma membrane approximately 50 Å in thickness and separated from the adjacent cell

by an intercellular space of approximately 100 Å (Fig. 12). The stichosome is enclosed by a thick membranous structure. This structure consists of many fine membranes comprising the pseudocoelomic membranes but it also encloses the esophagus, separating the latter from the stichocyte (Fig. 22).

The nucleus was studied in the stichocytes of T. muris only. The spherical nucleus is found near the center of the cell. It is enclosed by a double-layered envelope. Each layer is approximately 70 Å in thickness and separated from the other by a space of 130 Å. Pores spaced at intervals of 0.1 micron occur in the nuclear envelope (Fig. 15). Many small blebs are formed by outpocketings of the nuclear membranes (Figs. 14, 15, 16). They extend into the cytoplasm for a distance of 1.0 micron or less. Generally, the base of the bleb is smaller in diameter than the bleb itself. Occasionally the outer portion of the bleb is turned at right angle simulating a hook-like structure. In the cytoplasm adjacent to the nucleus there are numerous small areas which are enclosed by a double membrane. The latter is similar to the nuclear membrane in thickness and also has pores (Fig. 15). The material contained within the membrane appears identical with the homogeneous granular nucleoplasm. The small enclosed areas represent either cross-sections of hook-like blebs in which the base does not appear or blebs which have been pinched off from the nucleus. The nucleolus is a large structure located near the center of the nucleus (Fig. 14). It is an electron-

dense homogeneous area. Within the nucleolus there are numerous regions which have an electron density similar to that of the nucleoplasm.

The cytoplasm of the stichocyte contains a large number of granules presumably secretory in nature. In electron micrographs the granules appear as oval bodies of various sizes up to 0.8 micron (Figs. 14, 18, 19). All of the granules have their long axis oriented in the same direction. This is suggestive of compression which occurred during sectioning and distorted the normal spherical shape of the granules. Internally, the granules appear to consist of a dense concentration of particulate matter.

Mitochondria are found closely associated with the secretory granules (Figs. 18, 19). The mitochondria appear as oval or round bodies with a dense matrix and many tubular cristae (Fig. 17). The cristae are distinct in some mitochondria but are almost obscured by the dense matrix of others. The latter are very similar in size and density to the secretory granules (Figs. 18, 19). Mitochondria also occur at the periphery of the cell. In T. muris, they are pleomorphic and have bizarre shapes. In T. vulpis they usually are spherical (Fig. 22).

Vesicles of various sizes up to approximately 0.4 micron occur scattered in the cytoplasm (Fig. 18). A limiting membrane of about 75 Å encloses an area of loose fine particulate substance. A system of canaliculi is often seen between two vesicles. In section the

canaliculi have many shapes and occasionally resemble a series of small vesicles which are in the process of coalescence. The canaliculi are continuous with the above mentioned large vesicles and contain the same particulate substance (Fig. 20). They are present in the region of the esophagus and may serve as a duct system for cell products to enter th digestive tract (Fig. 23). No opening of the canaliculi into the esophageal lumen has been observed. However, vesicles containing the previously mentioned particulate substance have been seen in the cytoplasm of the cell surrounding the esophageal lumen (Fig. 24). In all sections studied the membranes separating the esophagus from the stichocyte have been intact with no openings allowing communication between the two structures. This does not eliminate however the possibility of there being such a connection.

The endoplasmic reticulum of the stichocyte is distributed throughout the cell (Figs. 12, 16). The rough-surfaced membranes of the endoplasmic reticulum are approximately 55 Å in thickness. RNP particles ranging in size from 75 to 100 Å are attached to the membranes (Fig. 21). These particles are densely concentrated throughout the cell. They often occur in parallel rows 150 Å apart. In some areas small whorls of cisternae and vesicles of endoplasmic reticulum are seen in cross-section. They are enclosed by a membrane approximately 55 Å in thickness.

The esophagus is partially or completely enclosed by the

stichocyte (Fig. 25). A membranous structure surrounds the esophagus and is continuous with the pseudocoelomic membranes which enclose the stichosome. The thickness of this membranous structure is variable. In some regions it appears as a single dense line. In other areas as many as six membranes have been observed. Each of these membranes has a thickness of 125 Å. The lumen of the esophagus generally is hexaradiate (Fig. 25). This condition is difficult to determine in sections where the lumen is dilated. In cross-section the lumen is enclosed by a single tubular esophageal cell (Fig. 25). The length of this cell has not been determined. The outer plasma membrane of the cell commonly has six invaginations. The membranes enclosing the esophagus are folded into these invaginations. The cytoplasm of the esophageal cell contains RNP-like particles and vesicles of endoplasmic reticulum. Small mitochondria occur infrequently. In various portions of the esophagus there are small fibrils approximately 100 Å in thickness extending perpendicular to the lumen wall (Fig. 26). These are believed to be contractile elements which dilate the esophageal lumen.

IV. DISCUSSION

The bacillary band

The term "bacillary band" has been used by various authors to indicate two separate structures. Some have considered the "band" to be an area of the cuticle penetrated by numerous rod-like structures. Others have used the term to designate both the cuticular area and its underlying hypodermal cells. In this study the bacillary band is considered to consist of the cuticular area containing the pores as well as the hypodermal cells which open into these pores. These cells are hypodermal in origin and constitute an enlargement of one of the lateral lines. However, their specialized structure warrants a distinct name. In this report, they have been called the bacillary cells.

The structure and function of the bacillary band have been studied by numerous investigators. It was first mentioned by Dujardin (1845). He described the bacillary band as a wide area consisting of papillae and granules swollen by endosmosis. Schneider (1866) corrected Dujardin's work by noting the presence of numerous fine rods which penetrated the cuticle. He also described the subcutaneous tissue as thick and covered by a thin layer of muscle. Eberth (1859) gave the first detailed description of the bacillary band. He considered the granular band (bacillary band) to be composed of round or polygonal shiny bodies in the cuticle. The

cuticular ridges present on the remainder of the worm were absent in this area although fine notches could be seen. The whole area was described as being transected by slender, closely packed, shiny rod-like bodies or plugs. The plugs corresponded to the granules of the cuticle (Dujardin, 1845) and in profile view might have appeared as papillae. Each plug consisted of a fine stem and a highly refractile flask-shaped swelling. In surface view each plug appeared as a fine point surrounded by a ring. Heine (1900) also described the bacillary band as consisting of fine rods which penetrated the cuticular layer. In longitudinal section the rods contained a fine fibrous substance and were mounted in cup-shaped holes in the cuticle. Bastian (1866) had already observed that the bacillary band consisted of many closely-packed cuticular pores. Jägerskiöld (1901) studied the bacillary band of several species and described it as a "Stäbchenfeld" (field of little rods). He reported hypodermal glands underlying the "Stäbchenfeld". Each hypodermal gland cell opened through a cuticular pore. Eberth (1859) reported the rods to be cuticular structures produced by the cells underlying the bacillary band. However, Jägerskiöld (1901) noted that a secretion was discharged through the pores. Since the nematodes under study did not possess the usual nematode excretory system, Jägerskiöld therefore assigned an excretory function to the bacillary band. Chitwood and Chitwood (1950) disagreed with Jägerskiöld's reasoning since some species possess both well-developed hypodermal glands and a distinct ventral excretory gland. Rauther (1918), in a detailed study, enlarged upon

Eberth's (1859) description of the cells underlying the bacillary band. He reported the presence of special elongated epithelial cells. These cells were described as alveolar in structure with a central nucleus. A delicate lamellar system was found between the cells. Other epithelial cells ("indifferent") were present among these special cells. The distal end of the special cell contained a homogeneous oval or bulb-shaped structure. Surrounding this structure was a darkly stained area. The cuticle was sunken in a funnel-shape forming a distinct pore for each special cell.

Electron microscopy has demonstrated the bacillary cells to be columnar in shape as described by Rauther (1918). The central nucleus and the homogeneous oval or flask-shaped structure at the distal end of the cell are also in accordance with his description. However, the presence of the "indifferent epithelial cells" of Rauther has not been verified in this study. All of the cells of the bacillary band have a similar structure and each opens through a cuticular pore. Alveolar structures have been identified but are not present in all cells. The lamellar system described by Rauther as separating the cells was not found. It is doubtful that the lamellar system described in the present study is that of Rauther's. The lamellar system described in this report has dimensions which are beyond the limits of resolution of the light microscope. The darkly stained area at the distal end of the cell probably corresponds to the lamellar system. This area might have stained deeply due to the numerous basophilic particles present between the lamellae.

Rauther (1918) described the bacillary cell as consisting of two parts: the cell proper and its secretory product. The electron micrographs demonstrate that the cell proper is separated from the secretory product by the folded plasma membrane. This infolding of the plasma membrane forms the lamellar system which was previously reported by Sheffield (1962) to be endoplasmic reticulum.

The submicroscopic components of the bacillary cell correspond to those described for other animal cells. Sjöstrand and Hanzon (1954) described the plasma membrane of the mouse pancreas cell as 60 Å in thickness. Sjöstrand and Rhodin (1953) studied the proximal convoluted tubule cells in the mouse and found the plasma membranes which separated the cells as 80 Å in thickness. The plasma membrane of the bacillary cell was found to be 70 Å thick. This agrees with the reports of previous authors.

Mitochondria have been identified in the bacillary cell and are similar to the mitochondria described by Palade (1952) and later authors. The typical mitochondrion according to Palade (1952) is surrounded by a double membrane. The inner membrane has folds which form ridges ("cristae mitochondriales") into the matrix of the mitochondrion. This matrix is devoid of structure. The occurrence of tubules rather than ridges in mitochondria was reported by Bradfield (1953). Kmetec, Miller and Swartzwelder (1962) described tubular cristae in the mitochondria of the muscle cells of Ascaris lumbricoides suis. The cristae in the mitochondria of the bacillary

cells appear to be of the tubular type. Occasionally circular profiles of the cristae are seen in cross sections of the mitochondrion.

The nucleus of the bacillary cell is enclosed by a double membrane. Discontinuities in the nuclear envelope have been reported by many investigators: Callan and Tomlin (1950), Anderson and Beams (1956), Bennett (1956), Wischnitzer (1958), and Miller, Swartzwelder and Deas (1961). The resolution obtained in the study of the bacillary cells was not sufficient to determine if these discontinuities actually represent pores.

Granularity of the ergastoplasmic sac (endoplasmic reticulum) was noted by Weiss (1953) and later reported by Sjöstrand and Hanzon (1954). The latter authors described particles of 150 Å on the membranes of the endoplasmic reticulum. Palade and Siekevitz (1956), using the ultracentrifuge, separated out the particles and biochemically determined that they contain ribonucleic acid. The granules reported in this study correspond in size, appearance and distribution to the particles of Palade and Siekevitz.

The endoplasmic reticulum was described in the cytoplasm of tissue culture cells by Porter et al. (1945) using the electron microscope. Palade and Porter (1952) studied the endoplasmic reticulum in sectioned tissue. They reported vesicles and canaliculi which formed a network throughout the cell. Sjöstrand and Hanzon (1954) noted that the endoplasmic reticulum of the mouse pancreas was approximately 40 Å in thickness. The endoplasmic reticulum

membranes in the bacillary cells are very fine and are only rarely discernable. Small vesicles limited by a fine membrane are occasionally seen. These are considered to be cross-sectional views of endoplasmic reticulum tubules in which the vertically oriented membrane provides sufficient density to record in the electron micrograph. Ribonucleic protein particles that are in parallel rows about 200 Å apart are suggestive of rough-surfaced endoplasmic reticulum.

Large dense granules have been noted in the bacillary cells of T. vulpis on several occasions. These granules are believed to be related to the secretory process in the cell. Dalton (1951) reported electron microscopic observation of the secretory granules of the pancreatic exocrine cells. These granules are spherical, very dense and average 0.6 micron in diameter. Although the granules described in the present study are smaller and less dense than those reported by Dalton, their occurrence in a cell that appears to be secretory in function is significant. The fact that they have only been observed in several cells of T. vulpis and never in T. muris suggests that they may be present only during a certain period of cellular activity. Studies of the bacillary cells in worms of various states of maturity may clarify the frequency of occurrence and the functional significance of these granules.

A structure resembling the lipochondria of the human eccrine sweat gland as reported by Iijima (1959) has been seen in the bacillary cells of T. vulpis. Iijima described the formation of

lipochondria from small granules originating in the Golgi vesicles. As the granules increased in size they became vacuolated and eventually disintegrated. Lacy (1953) also reported lipochondria in cells of the pancreas and thought that they might be involved in the secretory process. The lipochondria of the bacillary cell range in form from a small granule to a large vacuolated structure. They occur in the area of the Golgi apparatus and may be similar to the lipochondria of Iijima.

A function for the bacillary cells has been postulated by several authors. Eberth (1959) thought that the cells secrete the rod-like structures which appear in the cuticle of the bacillary band. As previously mentioned, Jägerskiöld (1901) assigned an excretory function to them but this was denied by Chitwood and Chitwood (1950). Müller (1929) approached the problem differently by considering the bacillary cells to be involved in the nutrition of the worm. This reasoning was partially based on the fact that the esophagus was not muscular and therefore could not draw in food. Chitwood and Chitwood (1950) reported muscle fibers in the esophageal wall and therefore disagreed with Müller. Based upon the present investigation, the bacillary cells seem to be secretory in function although the nature of the product secreted is not known. Several possibilities must be considered. The anterior portion of the worm is embedded in the intestinal mucosa of the host. Therefore, it is possible that the bacillary band may provide a tissue-destroying

substance to promote the migration of the worm. Nimmo-Smith and Keeling (1960) studied the hydrolytic enzymes of T. muris and concluded that they were probably concerned with the parasite's own metabolism rather than invasion of the host's intestinal mucosa. Another possibility is that suggested by Müller (1929). He thought that the bacillary cells secreted a substance which dissolved the host tissue. This same group of cells then absorbed the digest and transported it to the stichosome through the connecting membranes. It is unlikely the bacillary cells would transport materials in both directions simultaneously. Also, the connecting membranes between the stichosome and the bacillary band are supporting structures (Chitwood and Chitwood, 1950). A third possible function of the bacillary cells is excretion, which was postulated by Jägerskiöld (1901). Several electron microscope studies tend to support this view. Pease and Baker (1950) described "tubular sheaths" surrounding the mitochondria at the basal end of the mammalian proximal convoluted tubule cells. Later, Pease (1955) reported these sheaths to be infoldings of the basal plasma membrane and related the structure to the transport of water. Kurosumi (1961) considered the infolded basal plasma membrane to play a role in the absorption of water and water-soluble substances from the intracellular fluid surrounding a gland. Beams et al. (1955) described similar folding of the basal plasma membrane in the Malpighian tubule of insects as did Rasmont et al. (1958) in the coxal gland

of the scorpion. While the basal folding of the bacillary cell does not correspond exactly with that described for the kidney and other cells, it does provide an increased surface area for absorption of materials into the cell. Recently, Smith and Littau (1960) reported the structure of the Malpighian tubules of the leafhopper, Macrosteles fascifrons. In two areas of the tubule they observed cells which had infolded basal plasma membranes and a system of lamellae at the apical ends. They related these structures to those of the vertebrate kidney and suggested parallel functions of the two organs. In the Malpighian tubule cells the mitochondria are found distributed in the central region of the cell. In the vertebrate kidney (Pease, 1955) the mitochondria are in close association with the basal infolding of the plasma membrane. However, in the bacillary cells the mitochondria are found near the apical ends of the cells. Since mitochondria are considered to function as energy producers (Green and Hatefi, 1961), their position may suggest the most active region of the cell. The bacillary cells may perform as an osmotic regulator for the worm. The mitochondria may supply the energy necessary to transport materials to the exterior through the cuticular pores.

While the evidence is not conclusive, it is suggestive that bacillary cells are instrumental in removing waste materials from the worm rather than producing a specific secretory product.

The stichosome

Eberth (1859) reported the esophagus of Trichuris trichiura to be imbedded in a row of large glandular cells. Leuckart (1876) and Rauther (1918) also observed that the esophagus of the Trichuroidea was enclosed by large glandular cells. Bastian (1866) reported that the esophagus was devoid of the usual muscle fibers. These were replaced by granular cells. Müller (1929) considered the cells surrounding the esophagus to be related to the hypodermis. Chitwood (1930 studied the esophagus in Trichinella spiralis, Trichuris ovis and T. vulpis. He stated that the esophagus was surrounded by a membrane separating it from the "cell body". Later, Chitwood (1935) reported that the cells of the "cell body" were esophageal glands. He termed the individual cells "stichocytes" and the row of cells was called the "stichosome".

Electron microscopy of the stichocytes of T. muris and T. vulpis shows that the esophagus is either partially or completely surrounded by the stichocyte. The membrane enclosing the esophagus was noted by Chitwood (1930) to join the membrane which surrounded the stichocyte. Chitwood and Chitwood (1950) termed the membranes that cover and support the organs of the pseudocoelomic cavity "pseudocoelomic membranes". Beckett and Boothroyd (1961) reported the presence of a nucleated protoplasmic sheath around part of the esophagus in the larval stage of Trichinella spiralis. This sheath was not observed to cover the stichosome. The description of the

sheath and the accompanying electron micrographs are not clear enough to identify this structure. Unless the authors were describing the cell surrounding the esophageal lumen, no structure comparable to a protoplasmic sheath has been noted in T. muris or T. vulpis. Kmetec et al. (1962) observed pseudocoelomic membranes covering and separating the muscle cells in an electron microscopic study of Ascaris muscle. In the present study the esophagus was observed to be separated from the stichocyte by a membranous structure. A similar structure surrounds the stichocyte. These two join each other and may be the pseudocoelomic membranes.

The esophagus was described as a thin tube with a circular or oval lumen by Bastian (1866), Leukart (1876) and others. Heine (1900) recognized fibers in the esophagus. However, Müller (1929) considered the fibers to be supporting rather than contractile. He did not believe the esophagus to be nutritionally functional. Chitwood and Chitwood (1937) reported the presence of muscle fibers in the portion of the esophagus enclosed by the stichosome as well as in the anterior portion. He described the lumen to be subtriangular, triradiate or hexaradiate. In the present study, fibers, believed to be contractile in nature, were observed in various portions of the esophagus. The esophageal lumen usually is hexaradiate although it sometimes appears oval or circular due to dilatation.

The only previous study of the fine structure of the stichocyte is that of Beckett and Boothroyd (1961). They reported the presence

of a large round nucleus with a prominent nucleolus in the larva of Trichinella spiralis. The boundaries of the nucleus and nucleolus were described to be difficult to determine. In the present study of T. muris the nucleus is large, spherical and contains a dense nucleolus. The boundary between the osmophilic nucleolus and the nucleoplasm is distinct although no membrane was observed separating the two. The nucleus is surrounded by a nuclear envelope which consists of two osmophilic membranes which are penetrated by numerous pores. The nuclear envelope has many small blebs which extend into the cytoplasm.

Clark (1960) reviewed some of the literature concerning the transfer of nuclear materials into the cytoplasm. He indicated that three mechanisms have been previously described. 1. A bleb is formed at the nuclear membrane which then ruptures or is dispersed into the cytoplasm. 2. The nuclear membrane temporarily opens and materials pass out. 3. The materials diffuse out across the intact nuclear membrane. Clark reported the occurrence of large blebs on the nuclear surface in the pancreatic acinar cells of the rat. He also noted the occurrence of vesicles in the cytoplasm which appeared to be of bleb origin. The nucleoplasm was reported to have areas of dense material projecting into the bleb. A similar area of dense nuclear material in association with a bleb was seen in the stichocyte of T. muris (see Fig. 16). The blebs observed in T. muris may be related to transfer of nuclear material into the cytoplasm.

Numerous dense granules occur throughout the cell. These granules are assumed to be secretory in nature. Secretory granules were noted by Dalton (1951) in an electron microscopic study of the pancreatic exocrine cell. They were reported to be about 0.6 micron in diameter and very osmophilic. Challice and Lacy (1954) and Lever (1955) studied secretory granules and suggested that they may have arisen from mitochondria. In the stichocytes of T. muris mitochondria were found in close association with secretory granules. Often the mitochondria were very granular with few cristae. Many gradations between the usual mitochondrion and the dense secretory granule were observed. This may indicate a transformation of mitochondria into secretory granules.

The high prevalence of nuclear blebs may be correlated with the secretory nature of the stichocyte. The transfer of nuclear material by blebbing into the cytoplasm, the well-developed endoplasmic reticulum and the large number of secretory granules suggest a highly active secretory cell.

Eberth (1859), Bastian (1866) and Rauther (1918) reported intracellular canals in the stichocyte. Rauther observed openings into the esophagus in the first two or three cells. Chitwood and Chitwood (1937) found that each stichocyte had an opening into the esophagus. Richels (1955) reported the presence of a duct from the stichosome to the esophagus of Trichinella spiralis. In their study of the fine structure of the larva of T. spiralis, Beckett and Boothroyd (1961)

reported the presence of fine canaliculi in the cytoplasm of the stichocyte. They noted that the canaliculi were often seen near the esophagus but a duct was not found. A connecting duct between the stichocyte and the esophagus was not found in the present study. However, it is believed that some structural continuity may exist. Numerous large vesicles occur in the stichocyte both near the esophagus and in the vicinity of the secretory granules. Associated with these large vesicles are irregularly shaped canaliculi. The canaliculi are seen distributed throughout the cell and especially between the large vesicles. They are often observed near the esophagus as was reported by Beckett and Boothroyd (1961). The presence of the canaliculi, the large vesicles with which they are continuous and similar vesicles in the esophageal wall suggest that they form an intracellular system for transport of materials into the esophageal lumen.

V. CONCLUSION

Electron microscopy has shown the bacillary band and the stichosome of T. muris and T. vulpis to be similar in general to the descriptions by light microscopy of previous authors. The fine structure of the cells generally corresponds to that described by electron microscopists for other animal tissues. The fine structure of the bacillary band and the stichosome of T. muris and T. vulpis are similar regarding form and size.

The function of the bacillary band is probably secretory in nature. It has been postulated that the bacillary cells may serve an excretory function in the worm. The fine structure of the bacillary cell is somewhat similar to that of excretory cells in other animals.

The stichocyte is a secretory cell which may produce materials that are discharged into the esophageal lumen. No direct connection was observed but the frequent occurrence of vesicles and canaliculi in the vicinity of the esophagus is suggestive of a connecting system.

Physiological, histochemical and immunological studies of both the bacillary cells and the stichocytes may provide the necessary information to determine the actual function of these cells.

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VII. EXPLANATION OF PLATES

PLATE I

Figure 1. Longitudinal section of the cuticular pore in the bacillary band of T. muris. Selectron-methacrylate. Uranyl nitrate stain. 18,000X.

Figure 2. Longitudinal section of a pore containing the secretory product. T. muris. Selectron-methacrylate. Uranyl nitrate stain. 20,000X.

PLATE II

Figure 3, Cross section of T. muris. Note hypodermis (arrows) separating muscle cells from cuticle. Methacrylate. 6,900X.

Figure 4. Oblique section of the bacillary cells of T. muris. Cells are cut at different levels showing basal folding, nucleus, mitochondria and lamellae. Selectron-methacrylate. Uranyl nitrate stain. 10,700X.

PLATE III

Figure 5. Cross section of T. vulpis showing basal end of bacillary cells. Methacrylate. 7,800X.

Figure 6. Longitudinal section of the edge of a bacillary cell of T. vulpis. Note folding of plasma membrane into lamellar system (arrow). Methacrylate. 13,000X.

PLATE IV

Figure 7. Cross section of several bacillary cells of T. muris. Note mitochondria between lamellae and around periphery of cell. Selectron-methacrylate. Uranyl nitrate stain. 25,000X.

Figure 8. Longitudinal section of the bacillary cells of T. muris. Note cristae in unusually long mitochondrion. Selectron-methacrylate. Uranyl nitrate stain. 28,700X.

PLATE V

Figure 9. Cross section of the bacillary cells of T. muris. Note cross and longitudinal sections of endoplasmic reticulum tubules. Selectron-methacrylate. Uranyl nitrate stain. 15,900X.

Figure 10. Longitudinal section of the bacillary cells of T. vulpis showing dense secretory granules in basal portion of cell. Methacrylate. 7,600X.

PLATE VI

Figure 11. Longitudinal section of the bacillary cell of T. vulpis showing several stages of development of lipochondria. Methacrylate. 20,700X.

Figure 12. Longitudinal section of stichosome showing plasma membranes separating two stichocytes. Note extensive endoplasmic reticulum in both cells. Selectron-methacrylate. Uranyl nitrate stain. 25,600X.

PLATE VII

Figure 13. Cross section of T. muris showing the pseudocoelomic membranes around the stichocyte which partially encloses the esophagus. Methacrylate. 13,800X.

Figure 14. Cross section of the stichocyte of T. muris showing nuclear blebs. Selectron-methacrylate. Uranyl nitrate stain. 10,500X.

PLATE VIII

Figure 15. Cross section of nuclear envelope of stichocyte of T. muris. Note pores (arrows) in both nuclear envelope and bleb. Selectron-methacrylate. Uranyl nitrate stain. 25,800X

Figure 16. Longitudinal section of the stichocyte of T. muris showing bleb in nuclear envelope. Note granular area in the nucleoplasm adjacent to bleb. Selectron-methacrylate. 34,300X.

PLATE IX

Figure 17. Cross section of stichocyte of T. vulpis showing mitochondria with tubular cristae in cross section (arrow). Methacrylate. 32,500X.

Figure 18. Longitudinal section of stichocyte of T. muris showing vesicle and nearby canaliculi. Selectron-methacrylate. 19,300X.

PLATE X

Figure 19. Longitudinal section of stichocyte of T. muris. Note similarity of secretory granules and mitochondria. Selectron-methacrylate. 19,000X.

Figure 20. Longitudinal section of stichocyte of T. muris showing continuity between vesicle and canaliculi. Selectron-methacrylate. 27,200X.

PLATE XI

Figure 21. Section of stichocyte of T. muris showing endoplasmic reticulum and attached RNP particles. Methacrylate. 48,000X.

Figure 22. Cross section of T. vulpis stichocyte showing pseudocoelomic membranes enclosing esophagus. Methacrylate. 8,900X.

PLATE XII

Figure 23. Longitudinal section of stichocyte of T. muris showing canaliculi near esophageal lumen and pseudocoelomic membranes in a depression of the cell surface. Selectron-methacrylate. 19,800X.

Figure 24. Section of stichocyte of T. muris showing vesicle within the esophageal cell. Selectron-methacrylate. 8,200X.

PLATE XIII

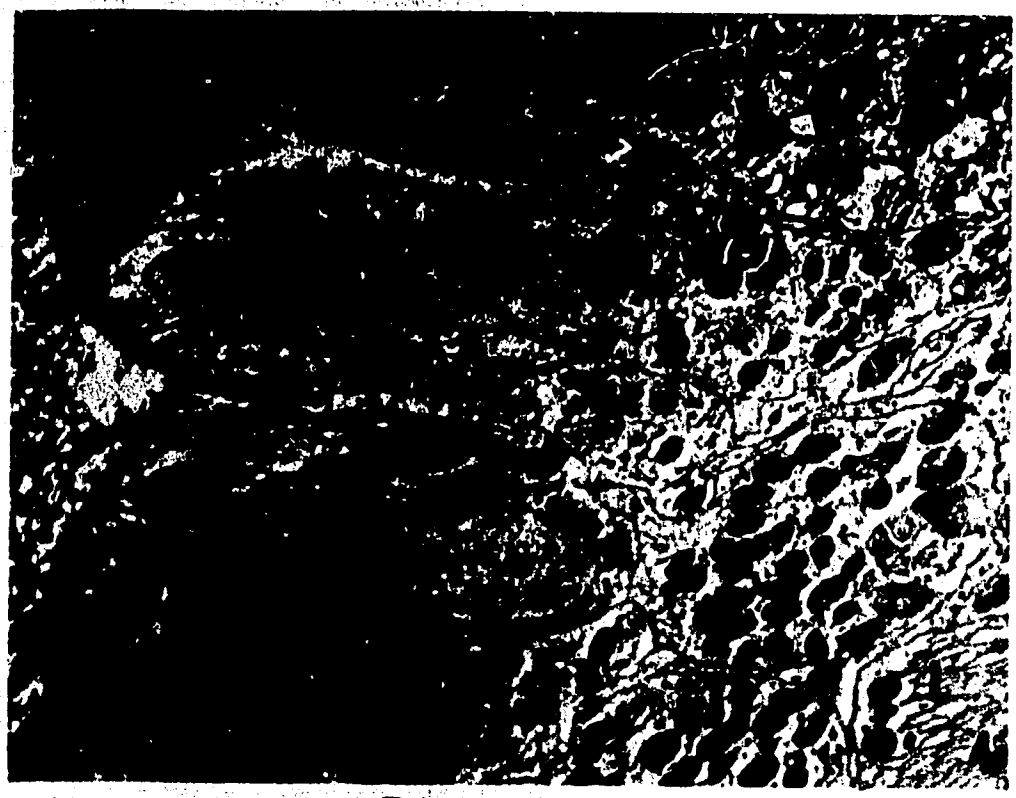
Figure 25. Cross section of stichocyte of T. muris showing hexaradiate lumen and tubular esophageal cell whose edges meet at arrow. Methacrylate. 9,900X.

Figure 26. Longitudinal section of stichocyte of T. muris showing an area of the lumen surrounded by an esophageal cell containing myofibrils. Cell edges meet at arrow. Selectron-methacrylate. Uranyl nitrate stain. 14,200X.

KEY TO SYMBOLS

B - Bleb
BC - Bacillary cell
BM - Basal membrane
C - Cuticle
CN - Canaliculi
E - Esophagus
EL - Esophageal lumen
ER - Endoplasmic reticulum
G - Golgi apparatus
GL - Glycogen
L - Lamellar system
LI - Lipochondrion
M - Mitochondrion
MC - Muscle cell
MF - Myofibrils
N - Nucleus
NE - Nuclear envelope
NU - Nucleolus
PC - Pseudocoelomic membranes
PM - Plasma membrane
S - Stichocyte
SG - Secretory granule
SP - Secretory product
V - Vesicle

PLATE I



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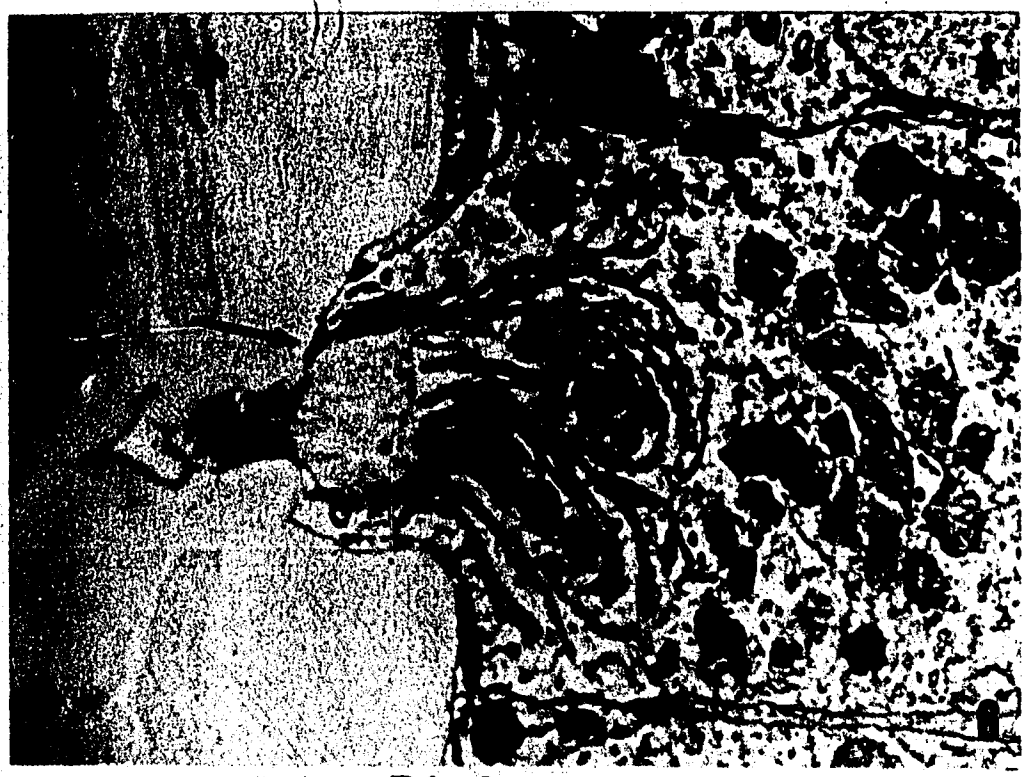
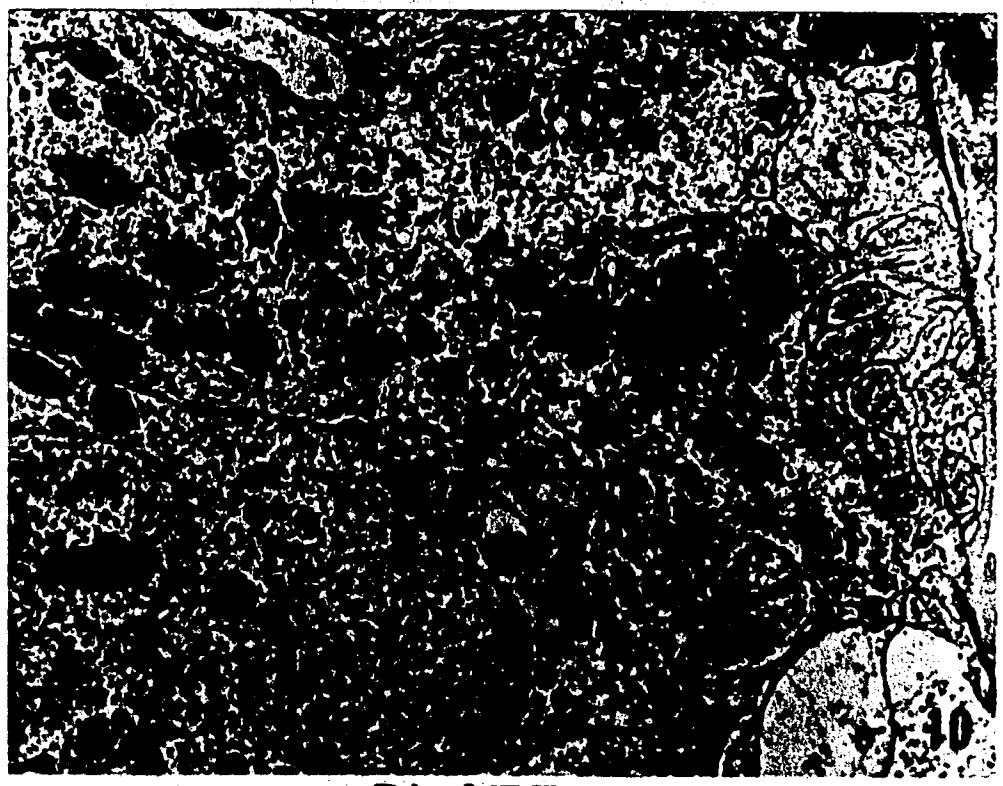


PLATE III

PLATE IV



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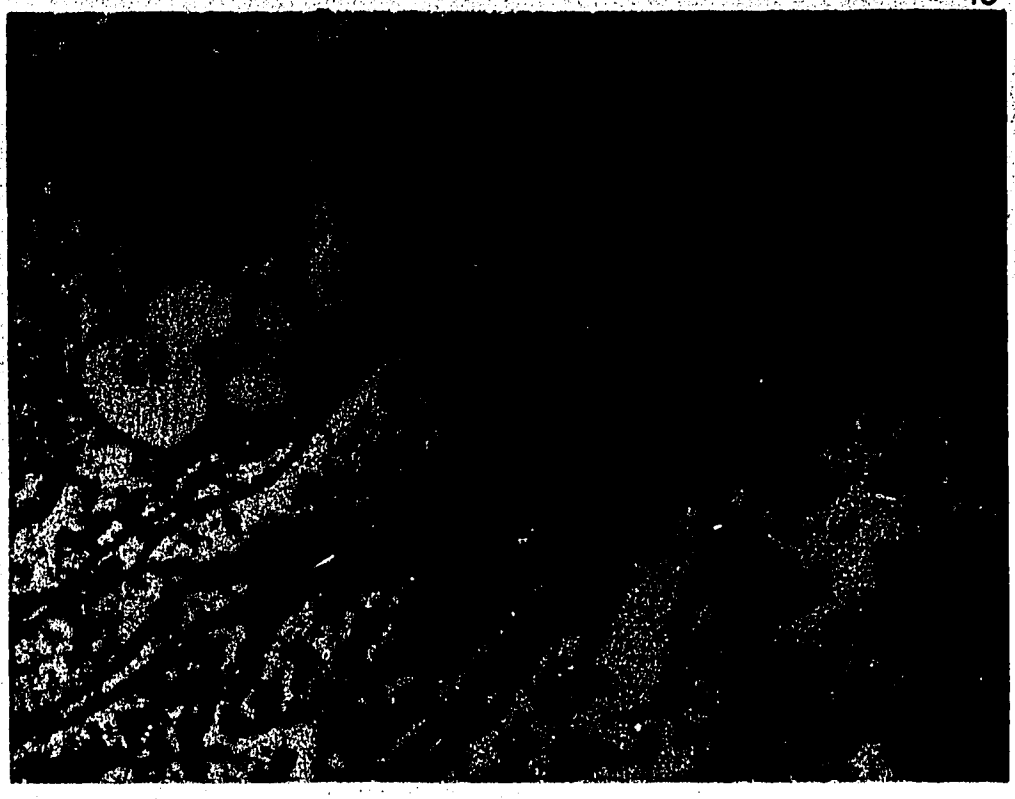
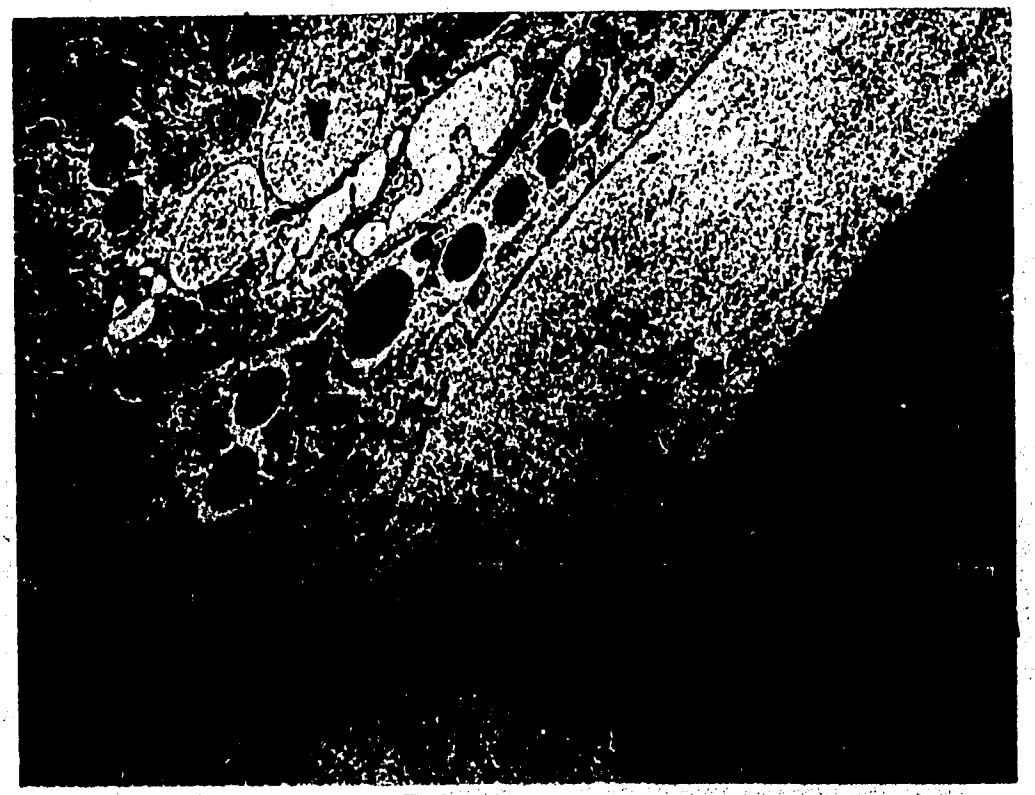


PLATE VI



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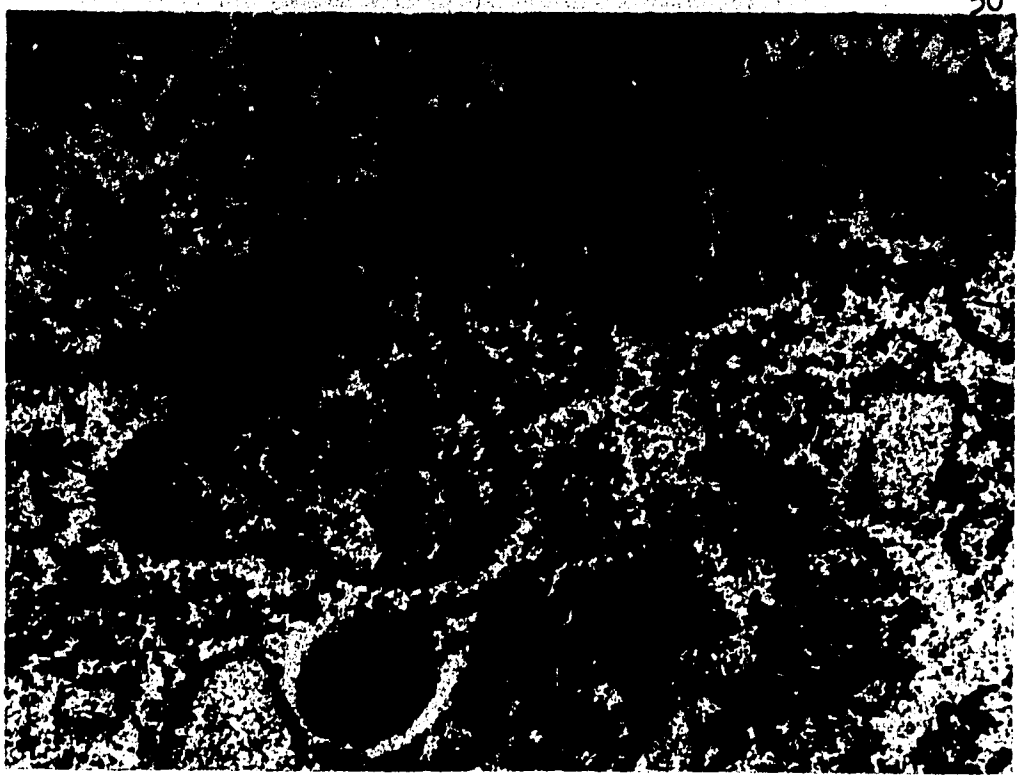

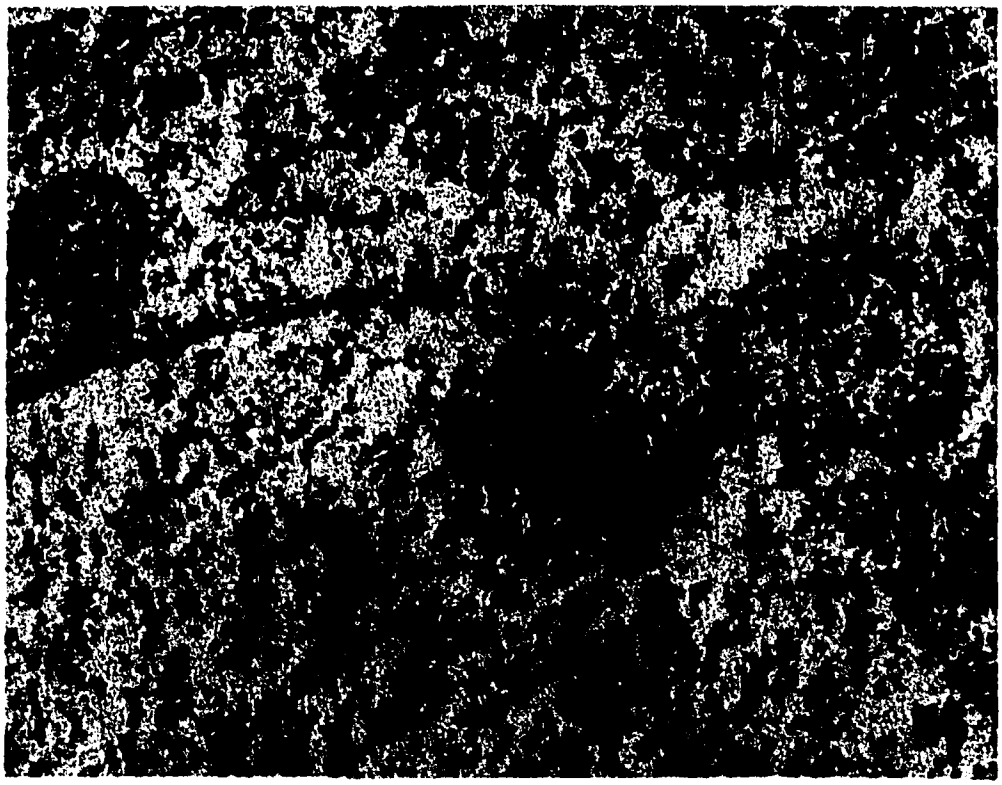

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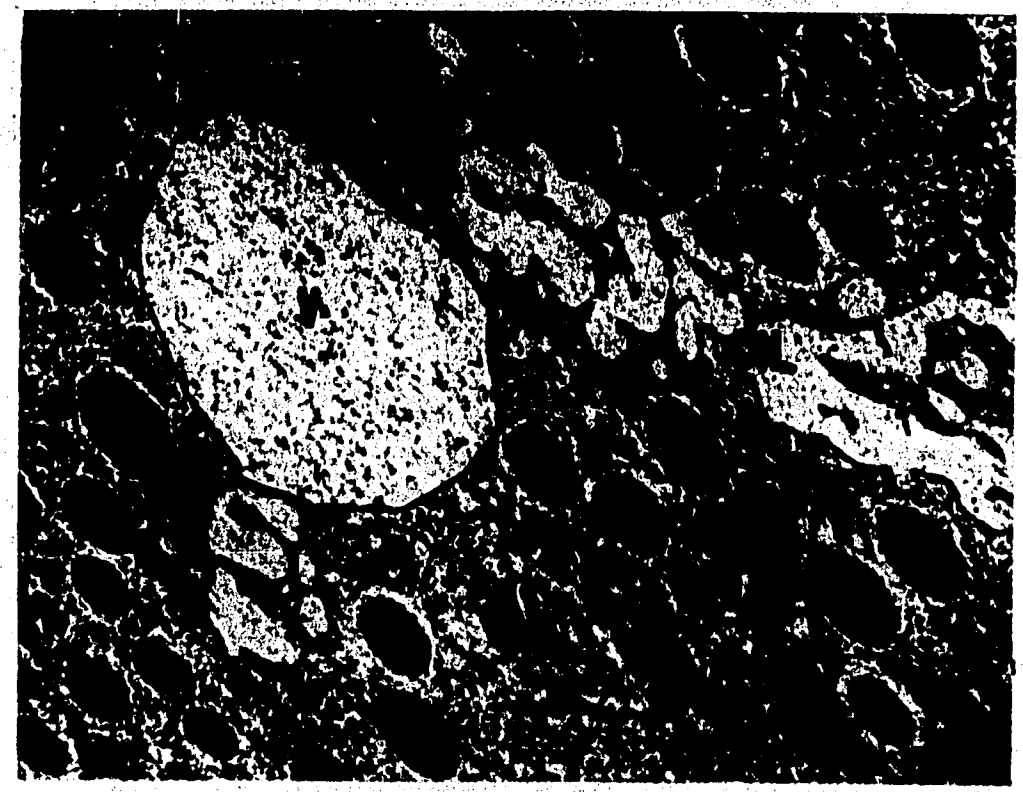

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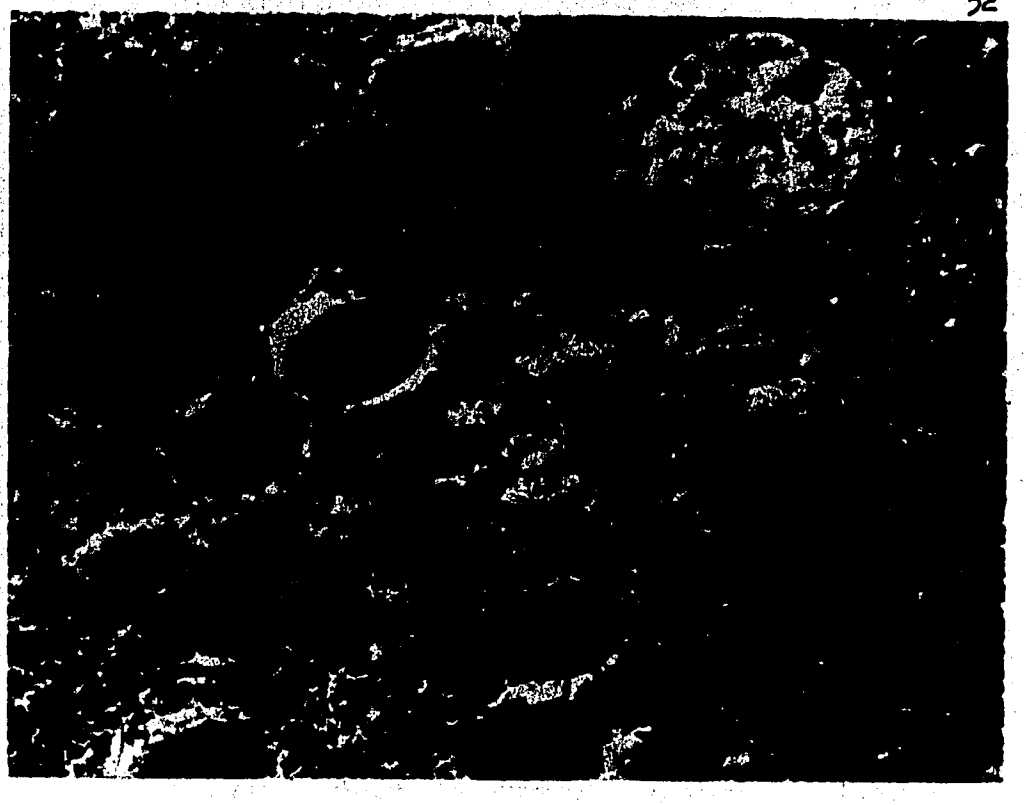


PLATE X

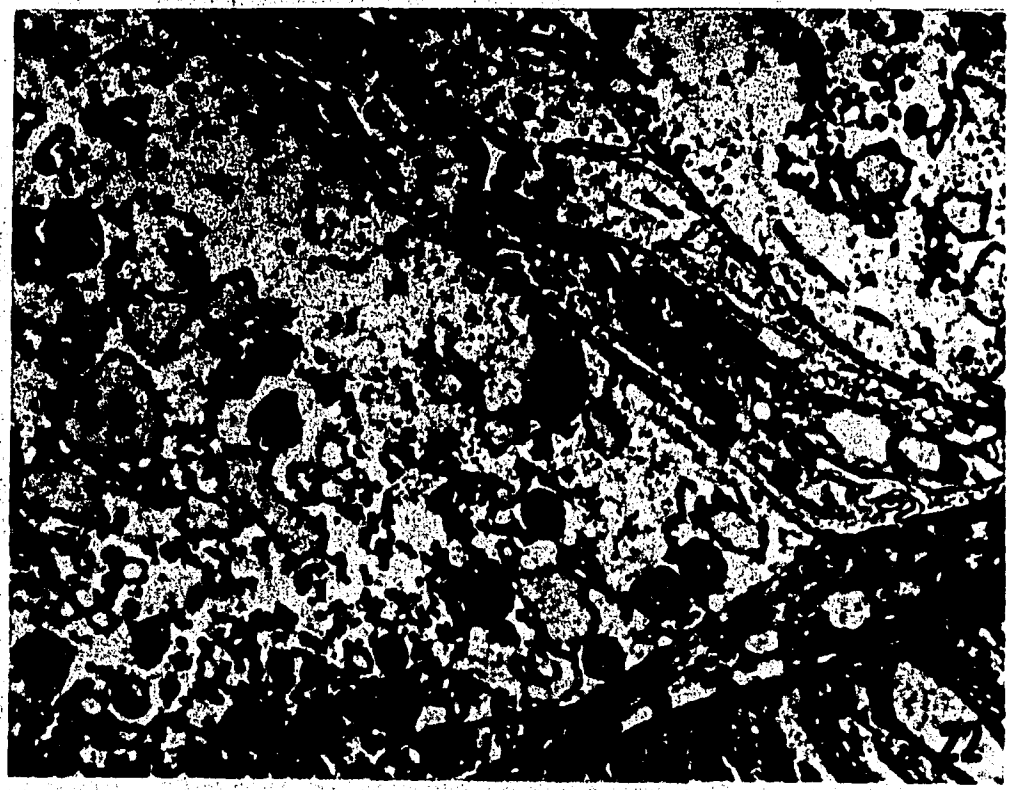
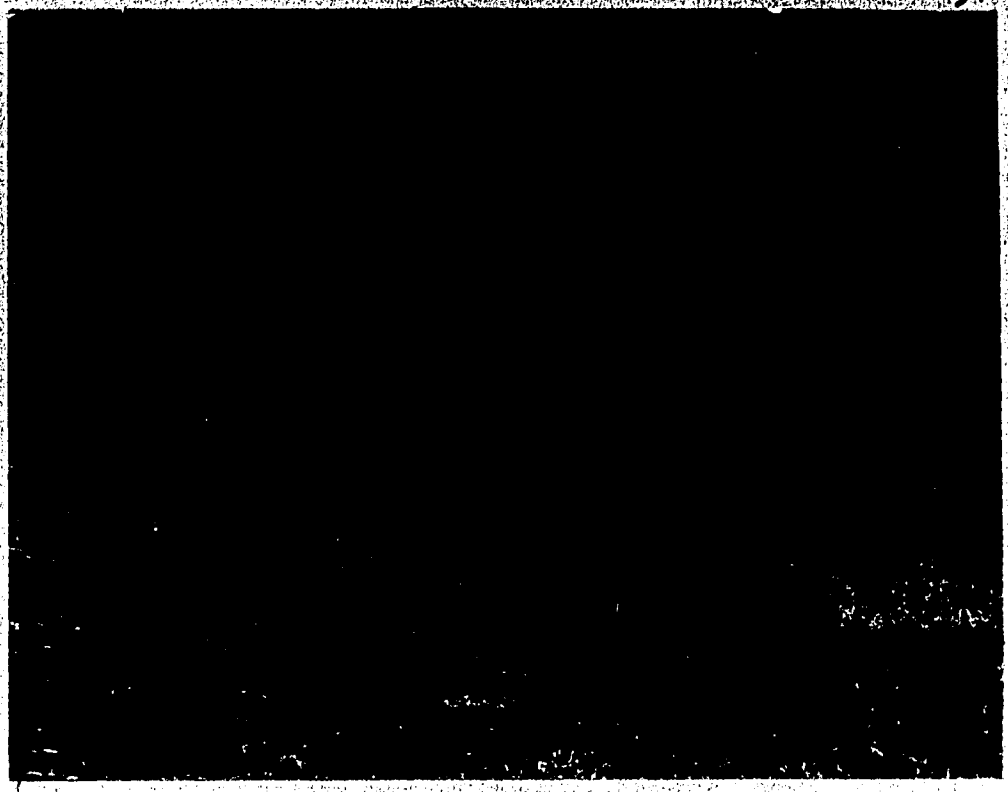


PLATE XI



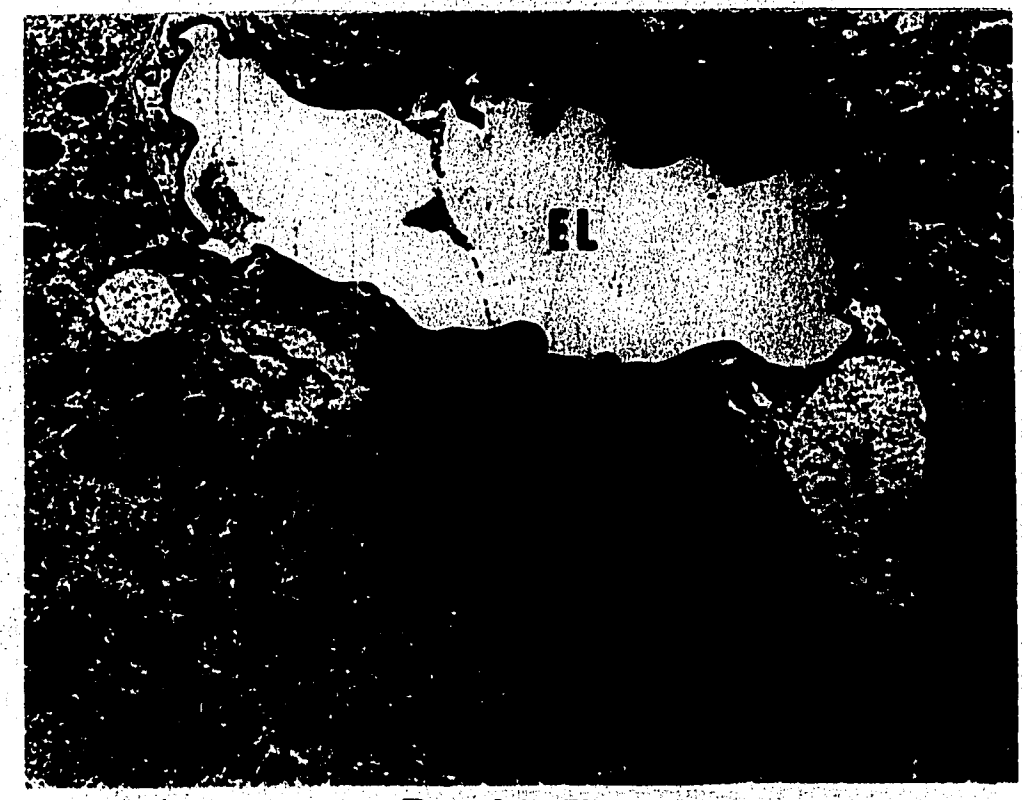


PLATE XII

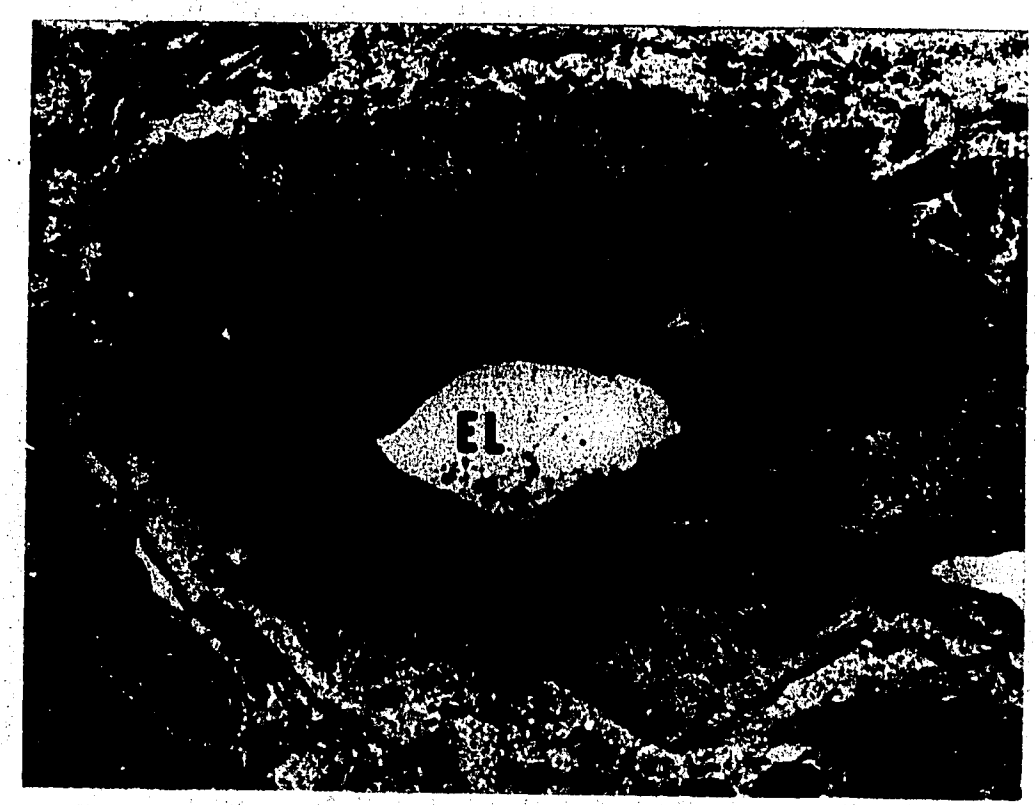


PLATE XIII

IX. BIOGRAPHY

Harley G. Sheffield was born in Detroit, Michigan, on January 10, 1932. He was graduated from Mackenzie High School, Detroit, Michigan, in January, 1949, and from Wayne State University in 1953 with a Bachelor of Science with a major in chemistry. Mr. Sheffield served as a microwave radio technician in the U. S. Army from 1953 to 1955. After release from the Army he entered Wayne State University Graduate School and received a Master of Science degree with a major in biology in 1958. He attended the University of Michigan Biological Station in the summer of 1957 and the State University of Iowa the following fall. He worked as a research biologist for Parke, Davis and Co. from 1958 to the fall of 1959 when he entered Louisiana State University Graduate School. Mr. Sheffield is now a candidate for the Doctor of Philosophy degree in Medical Parasitology at the Louisiana State University School of Medicine.

EXAMINATION AND THESIS REPORT

Candidate: Harley George Sheffield

Major Field: Medical Parasitology

Title of Thesis: Observations on the Fine Structure of the Stichosome and Bacillary Band of Trichuris muris (Schrank, 1788) and Trichuris vulpis (Froelich, 1789)

Approved:

J. C. Dougherty
Major Professor and Chairman

Max Goodrich
Dean of the Graduate School

EXAMINING COMMITTEE:

E. John Buddingh

A. Sidney Jones

Frank M. Law

H. J. Thorner

Joseph A. Miller

Date of Examination:

July 13, 1962